

# The National Biodefense Analysis and Countermeasures Center

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Managed and Operated by BNBI for DHS

**National Bioforensics  
Analysis Center**

**Sept 21, 2007**

**James Burans, Ph.D.  
Director, NBFAC**



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# Anthrax Attacks - 2001

- Florida
- New York
- Washington, DC
  - Demonstrated the need for a coordinated and dedicated national capability for bioforensic analyses to support biocrime/bioterror investigations

## Confirmed Anthrax Cases

Following are cases of anthrax confirmed by the Centers for Disease Control and Prevention. Other suspected cases remain unconfirmed by the CDC. They include two workers at the New York Post and a second NBC worker.



ASSOCIATED PRESS  
**Stevens**



ASSOCIATED PRESS  
**Blanco**



ASSOCIATED PRESS  
**Fletcher**



REUTERS  
**Curseen**



ASSOCIATED PRESS  
**Nguyen**

VICTIM	KIND	LOCATION	CONFIRMED	STATUS
Bob Stevens, 63	Inhaled	American Media, Boca Raton	Oct. 4	Died Oct. 5
Erin O'Connor, 38	Skin	NBC in Manhattan	Oct. 12	Recovering
Boy, 7 months	Skin	ABC in Manhattan	Oct. 15	Recovering
Ernesto Blanco, 73	Inhaled	American Media, Boca Raton	Oct. 15	Left hospital
Claire Fletcher, 27	Skin	CBS in Manhattan	Oct. 18	Recovering
Teresa Heller, 32	Skin	West Trenton post office	Oct. 18	Recovering
Patrick O'Donnell, 35	Skin	Hamilton Township mail center, N.J.	Oct. 19	Recovering
Leroy Richmond, 57	Inhaled	Brentwood mail center, D.C.	Oct. 21	Hospitalized
Unnamed man	Inhaled	Brentwood mail center, D.C.	Oct. 22	Hospitalized
Thomas L. Morris Jr., 55	Inhaled	Brentwood mail center, D.C.	Oct. 23	Died Oct. 21
Joseph Curseen Jr., 47	Inhaled	Brentwood mail center, D.C.	Oct. 23	Died Oct. 22
Unnamed worker, 59	Inhaled	State Department mail center, D.C.	Oct. 25	Hospitalized
Unnamed woman, 56	Inhaled	Hamilton Township mail center, N.J.	Oct. 28	Hospitalized
Non-postal worker	Skin	Works near Trenton, N.J.	Oct. 29	Left hospital
Unnamed worker	Inhaled	Hamilton Township mail center, N.J.	Oct. 30	Left hospital
Kathy Nguyen, 61	Inhaled	Manhattan Eye, Ear & Throat Hospital	Oct. 30	Died Oct. 31

SOURCES: Centers for Disease Control and Prevention, staff and wire reports

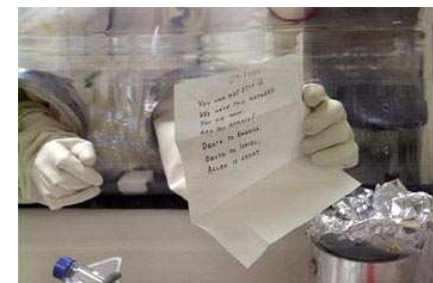


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# Bioforensic Investigative Challenges in 2001

- No dedicated biocontainment labs, staff or equipment to conduct bioforensic analysis
- No methods to enable handling of biothreat agent powders
- No methods to support traditional forensic analyses of evidence contaminated with a biothreat agent
- No place to receive large quantities or large pieces of evidence contaminated with a biothreat agent
- No established evidence handling, analytical methodologies, quality guidelines, peer review of methodologies or central coordination for bioforensic analyses



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# DHS Bioforensics Program

- Bioforensics research program
  - Short and Long term R&D to fill “capability gaps” in bioforensics to support the NBFAC
- National Bioforensics and Analysis Center (NBFAC)
  - Conducts casework
- An Integrated Program that enables:
  - Biological threat agent ID/characterization
  - Physical/Chemical analyses of matrices
  - Generate data for attribution analysis



# The Path Forward....

- BW Phylogenetics R&D late 1980's
- Navy/Army labs support UNSCOM 1993 – 1998
- Establishment of FBI HMRU 1996 – Microbial Forensics
- Establishment of FBI CBSU 2003
- Establishment of NBFAC by DHS 2004

***NBFAC will provide a national capability to conduct and coordinate forensic analyses of evidence from biocrime and bioterror investigations***

*HS - Presidential Directive 10, Biodefense for the 21st Century, April 2004*



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# NBFAC Strategic Goals

- Establish/maintain dedicated containment laboratories, specialized Spoke laboratories, and trained staff to conduct and coordinate bioforensic analyses
- Evaluate and establish assays for high consequence, human, animal, and plant biological threat agents
- Establish/maintain international accreditation for bioforensic casework (ISO 17025) and continue to expand the scope of accreditation for newly established capabilities
- Establish/maintain reference collections of biological agents with geographically and temporally diverse isolates, extracted nucleic acids, and antigens for comparative forensic identifications

# NBFAC Execution is Based on a Requirements-Driven Plan



Requirements established through DHS and:

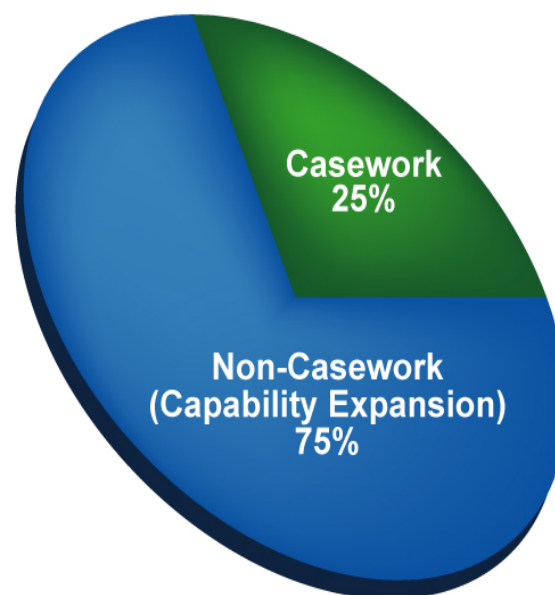
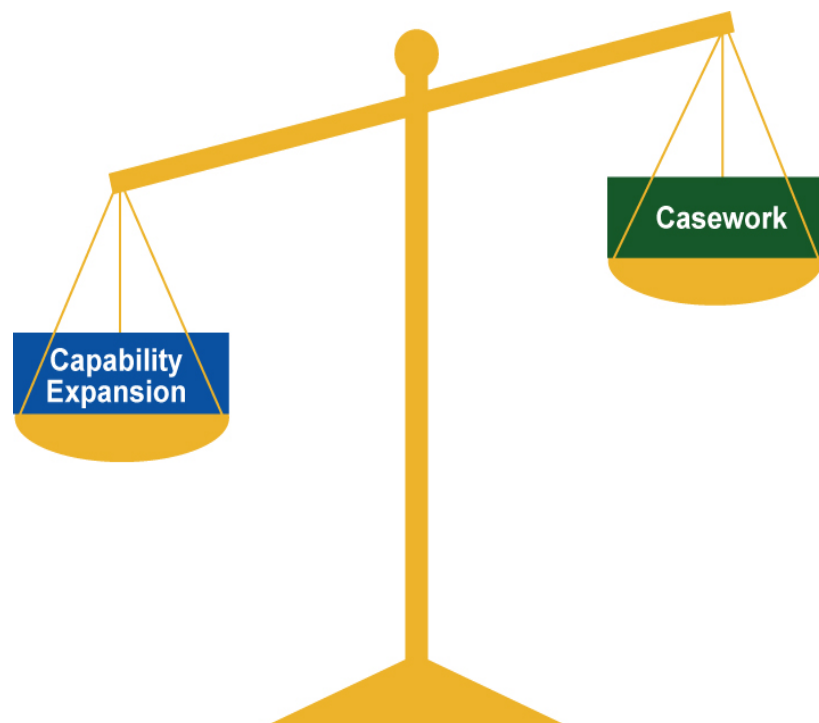
- FBI Scientific Working Group for Microbial Forensics (SWGMEF)
  - Semi-annual meetings of Subject Matter Experts (SMEs) and Users
  - Sample management, molecular signatures, chem/phys signatures, repositories
- Cold Spring Harbor Symposia on Microbial Forensics
  - Annual technical meetings with SMEs
- Bioforensic Working Group
  - FBI, IC, USSS, US CBPS, DHS, DOS,
  - Quarterly meetings - identify gaps – leverage investment
- International
  - UK/CA/AUS/US Sweden International Bioforensic Working Group



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# NBFAC- Balance Between Casework and Capability Expansion

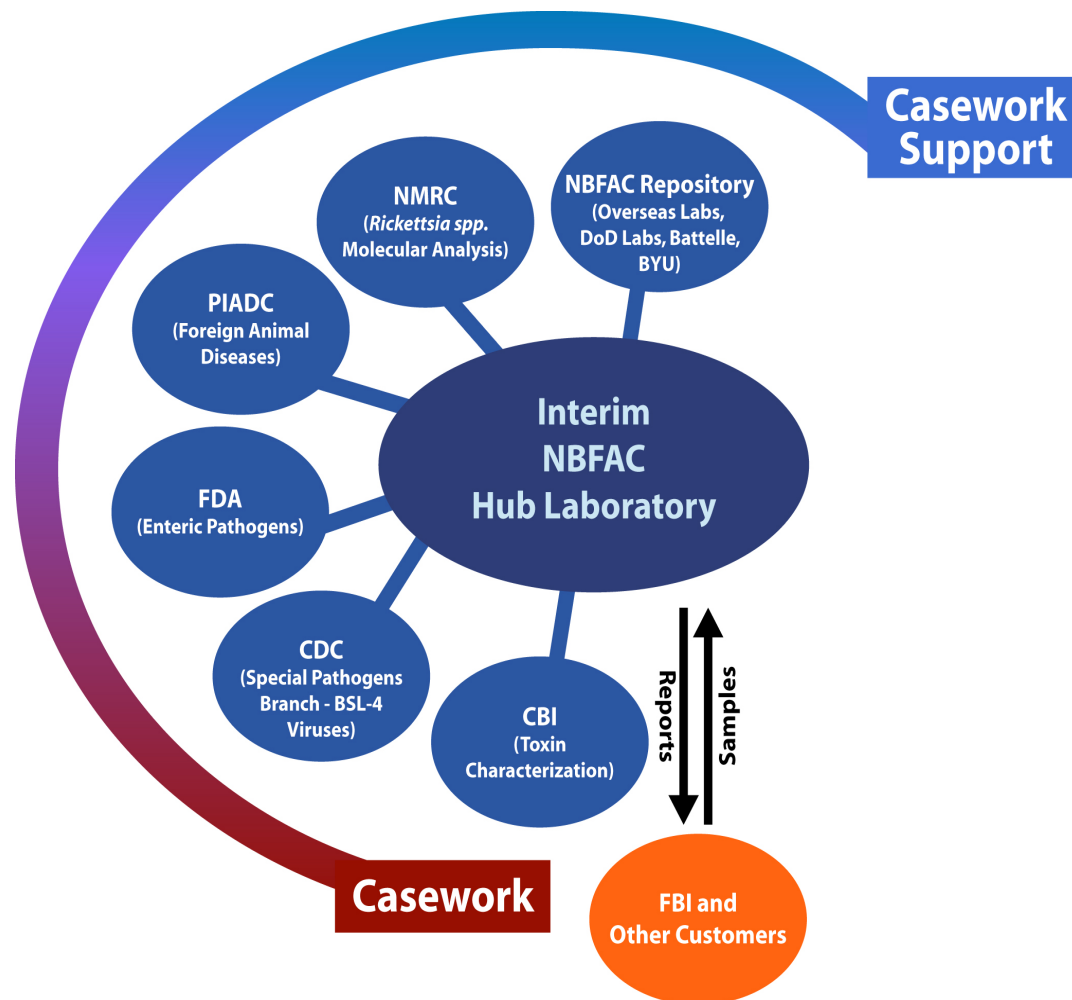


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# NBFAC “Hub” and “Spoke”

Provides a National Capability for Forensic Analyses  
Supporting Biocrime and Terror Investigations



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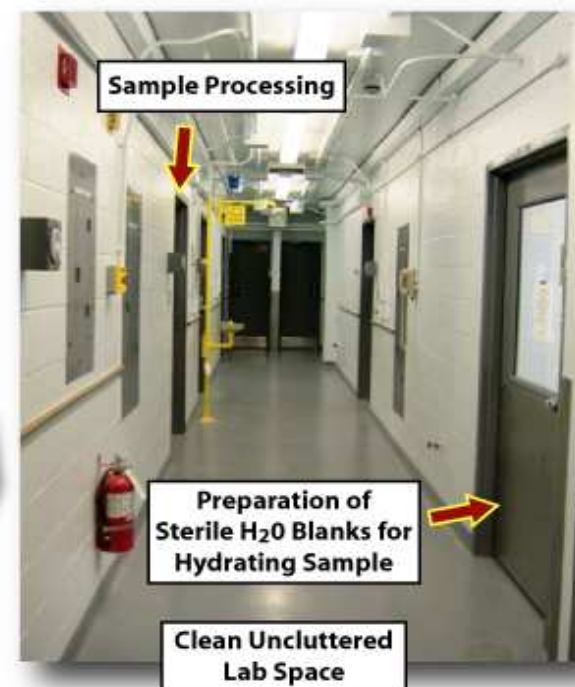
# NBFAC Contamination Control – Setting a New National Standard



- Total access control
- Segregation of processes
- Daily, weekly and monthly maintenance
- Daily and Weekly environmental surveillance for threat agent signatures



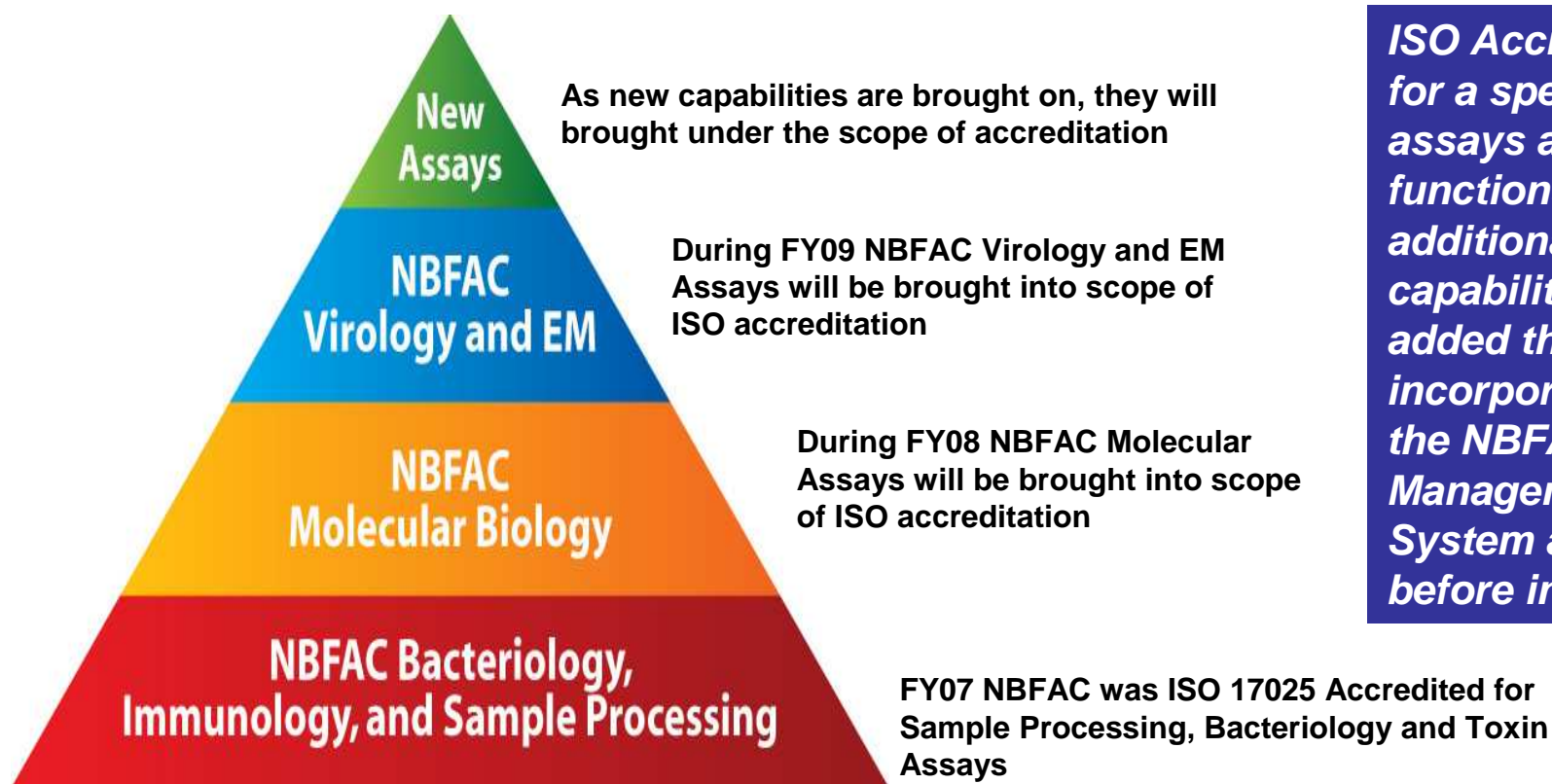
## Functional Dedicated Facility



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# NBFAC ISO 17025 Accreditation: Expansion of Scope



***ISO Accreditation for a specific set of assays and functions - as additional capabilities are added they must be incorporated into the NBFAC Quality Management System and audited before inclusion***

ISO 17025 Program standardizes and validates performance and competency of staff, equipment, reagents and assay results – continual external and internal assessment and improvement



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# NBFAC LIMS Phase II: A Complete End-to-End Solution For Bioforensics



Integrates Quality Management and Data Collection from Instrumentation



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# NBFAC Interim Hub Laboratories at USAMRIID

## *Interim* Solution with Dedicated Capability *Now*



2008



**New NBACC Bldg —  
11,000 ft<sup>2</sup> dedicated  
bioforensic casework —  
10,000 ft<sup>2</sup> for bioforensic  
support**

Sept 06



**3,000 ft<sup>2</sup> BSL-2 Molecular lab opens  
at NBFAC Interim Hub at USAMRIID**

Sept 05

**Bioforensic Spoke laboratory at Plum Island  
established for high consequence foreign animal  
disease agents**

May 04

**CDC select-agent-Certified BSL-2 and BSL-  
3 biocontainment laboratories open within  
USAMRIID**

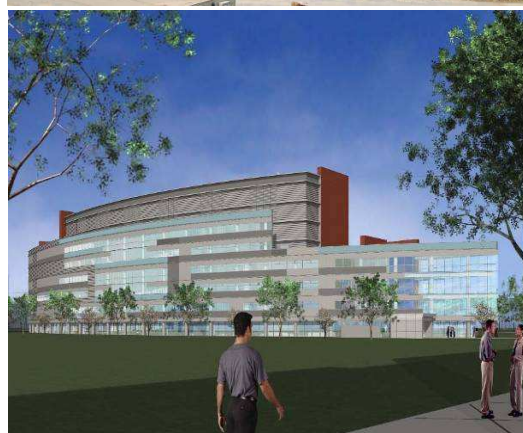


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# New NBACC Building



**10,000 sq ft BSL-2/3  
dedicated casework space**



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- Bio and signature containment design based upon “work-flow” and “types of analyses” to avoid live agent or signature cross contamination
- Casework analysis laboratories include: sample receipt/processing, bacteriology, virology, toxinology/immunology, molecular biology (MB) sample processing, MB PCR setup, MB amplification and post-amplification process areas
- Large “flexible bays” for large evidence receipt and use with law enforcement analytical equipment
- Separate casework support containment laboratories
- Individual casework analysis and support laboratories can be decontaminated with Vaporized Hydrogen Peroxide (VHP)





# NBFAC: Where we are now.....



NBFAC is an ISO 17025 Accredited Laboratory (now expanding scope)

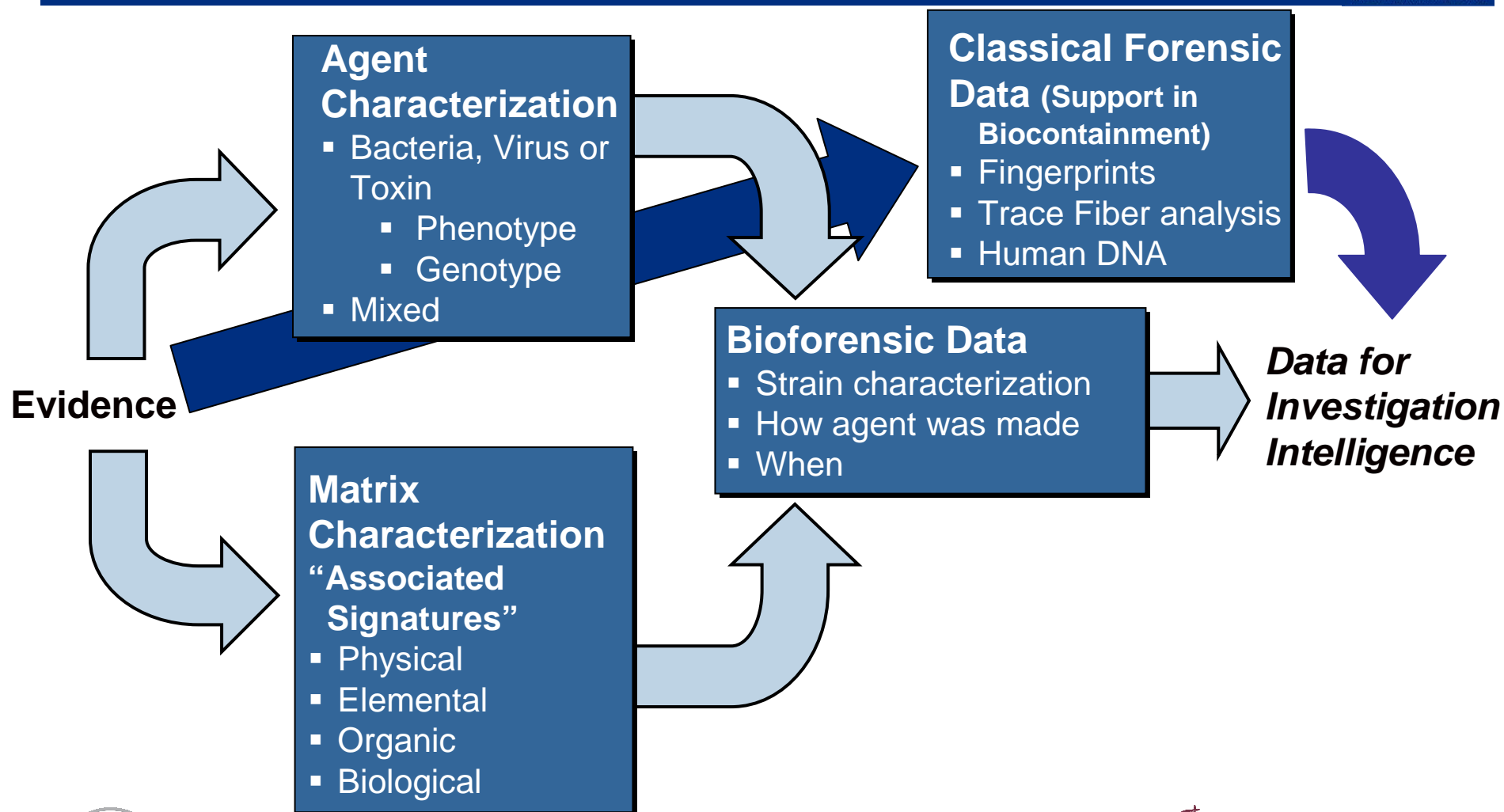
- Sample Processing – supports traditional forensics
  - LIMS Accessioning
  - Maintains chain of custody
  - Ships and receives samples
- Molecular Biology (BSL-2/3)
  - RT PCR
  - Genotyping
  - Sequencing/Bioinformatics
- Bacteriology (BSL-2/3)
  - Culture/enumeration
  - Phenotypic characterization
- Virology (BSL-2/3/4)
  - Culture/enumeration
  - Phenotypic characterization
- Toxinology/Immunology (BSL2/3)
  - Toxin identification
  - Characterize biological activity
- Foreign Animal Disease Bioforensic Spoke Laboratory at Plum Island, NY
  - Culture
  - Molecular analysis
- Repository Collection for Forensic Comparison (BSL2/3/4)
  - Live agent
  - DNA extracts
  - Agents prepared under different conditions and media



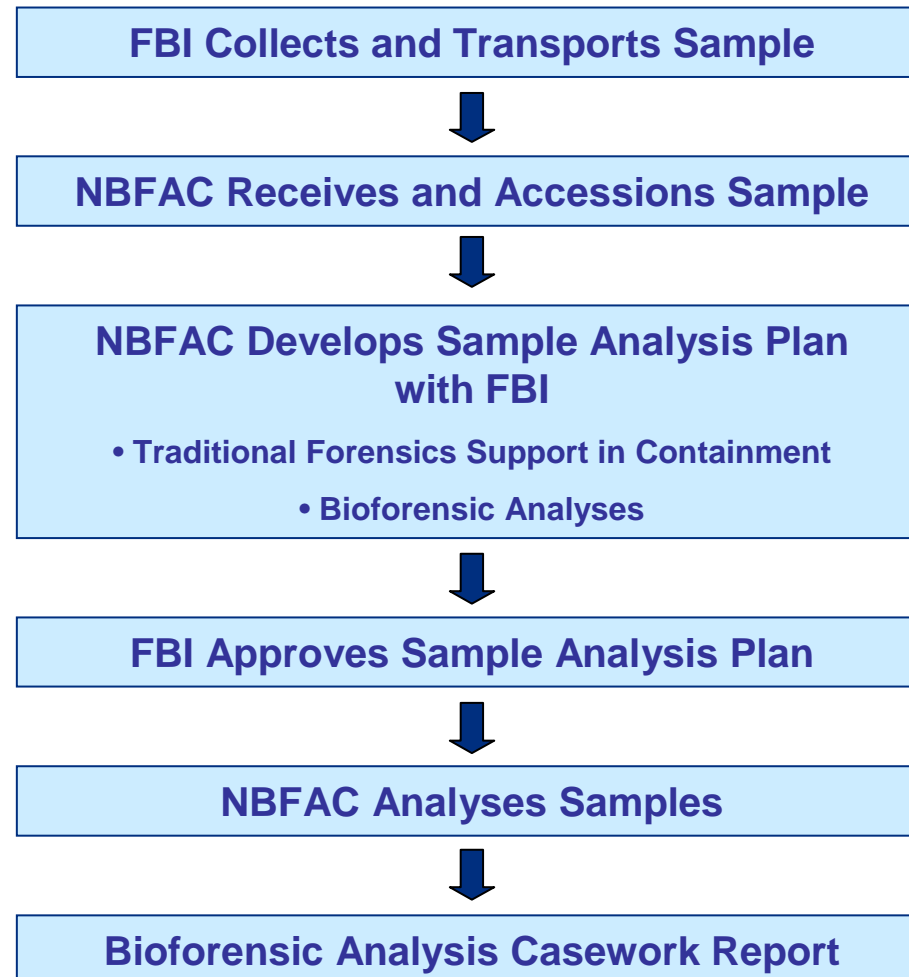
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# Bioforensic Analysis



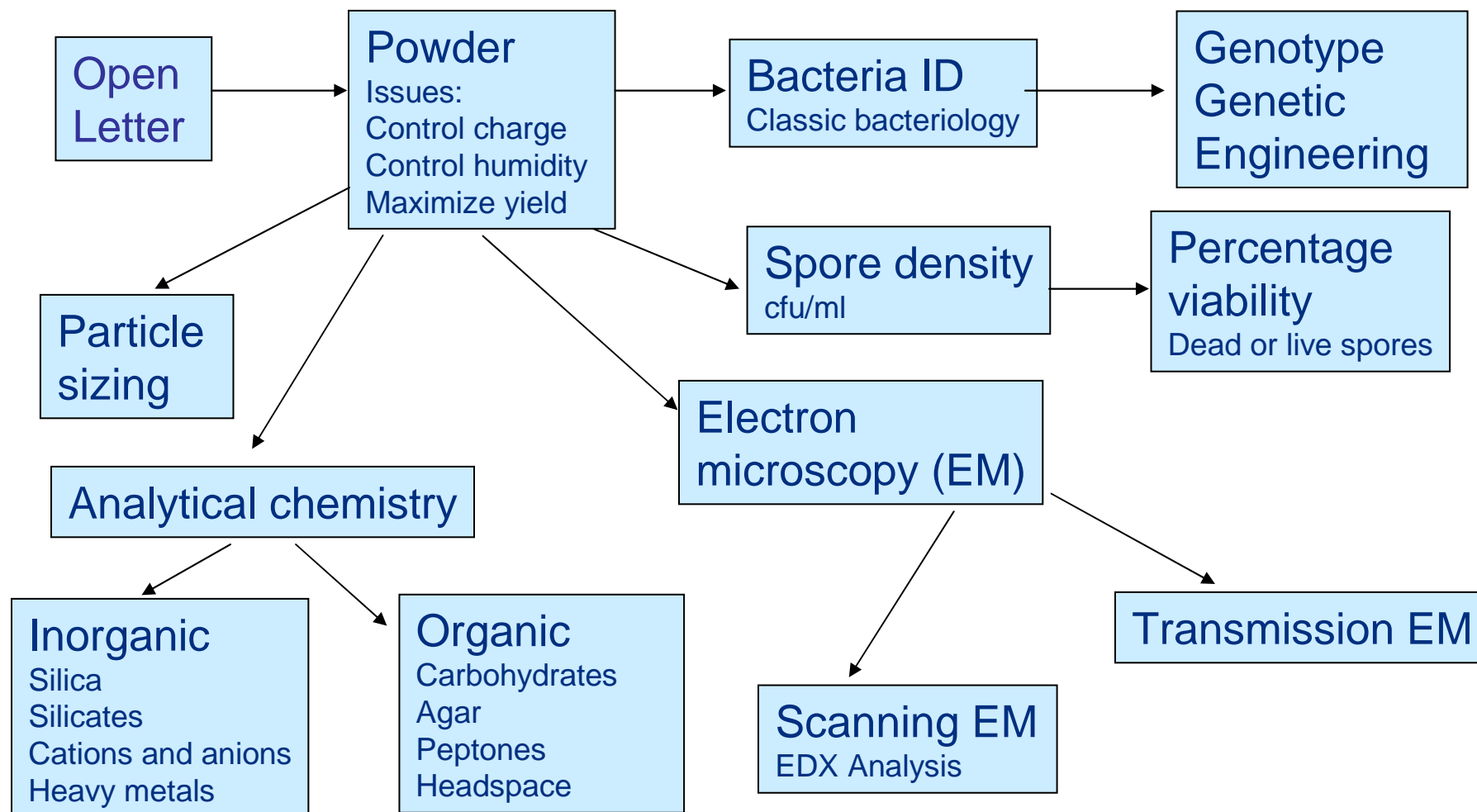
# Overview of NBFAC Casework Process



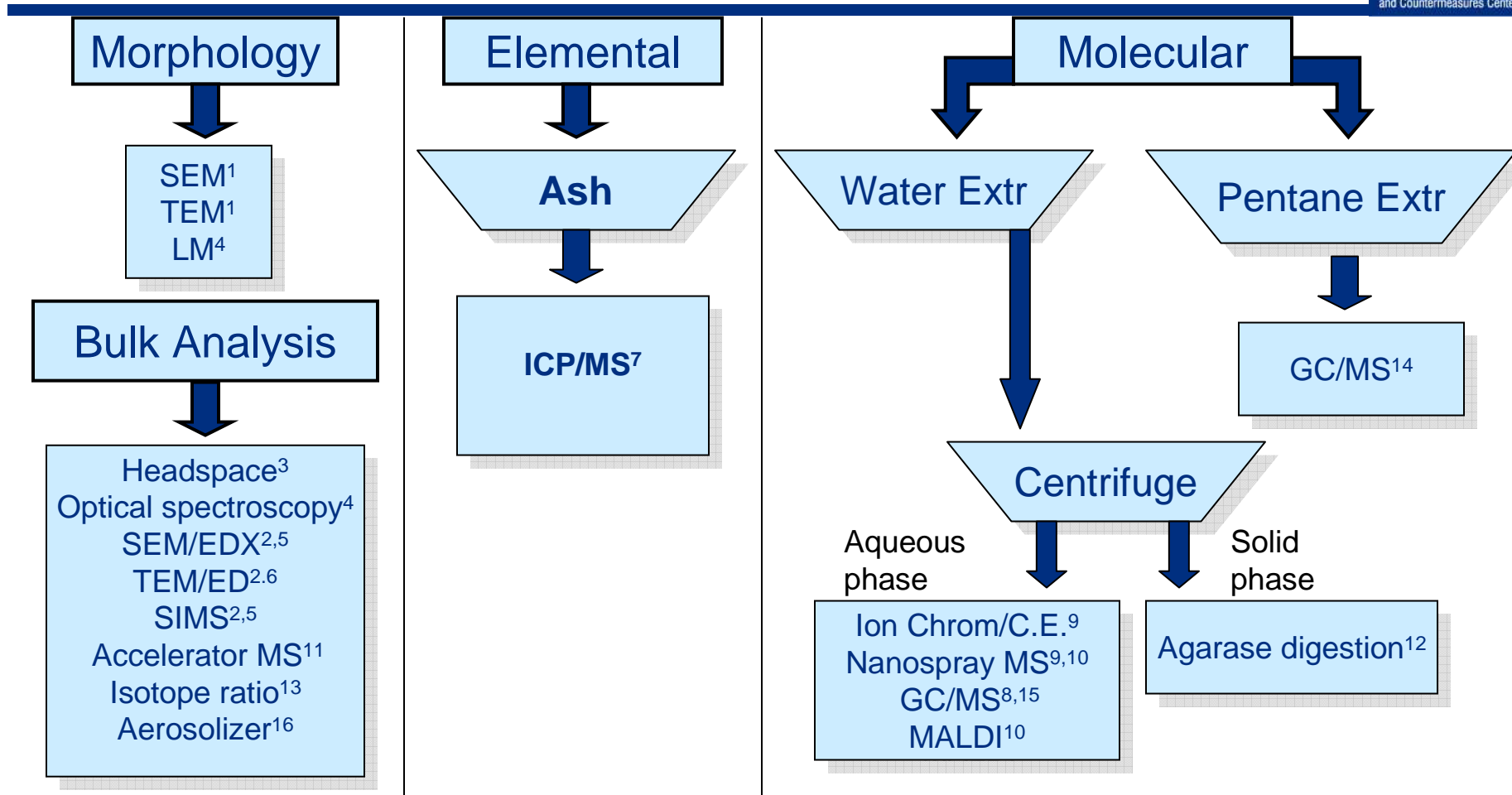
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# Sample Analyses Flow Chart



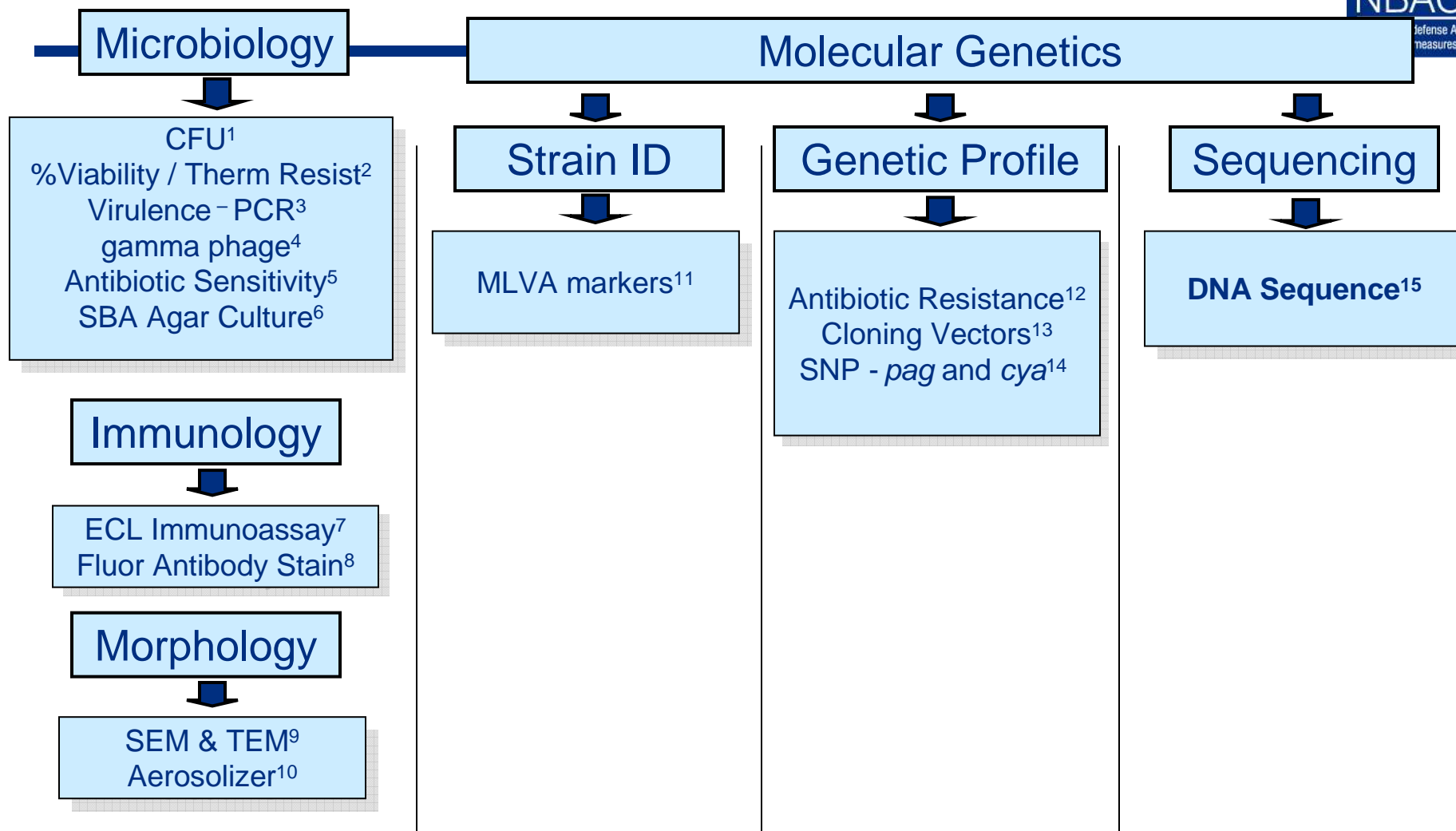
# Chemical and Physical Characterization of Bioforensic Samples



Anticipated Result	1 spore shape, quality	5 elemental mapping	9 buffer/media salts	13 geolocation
	2 silicon, silicates	6 crystal structure	10 peptide sequence	14 silicones
	3 drying solvents	7 elemental profile	11 age	15 polysaccharides
	4 phys/chem profile	8 simple sugars	12 agar determination	16 particle size

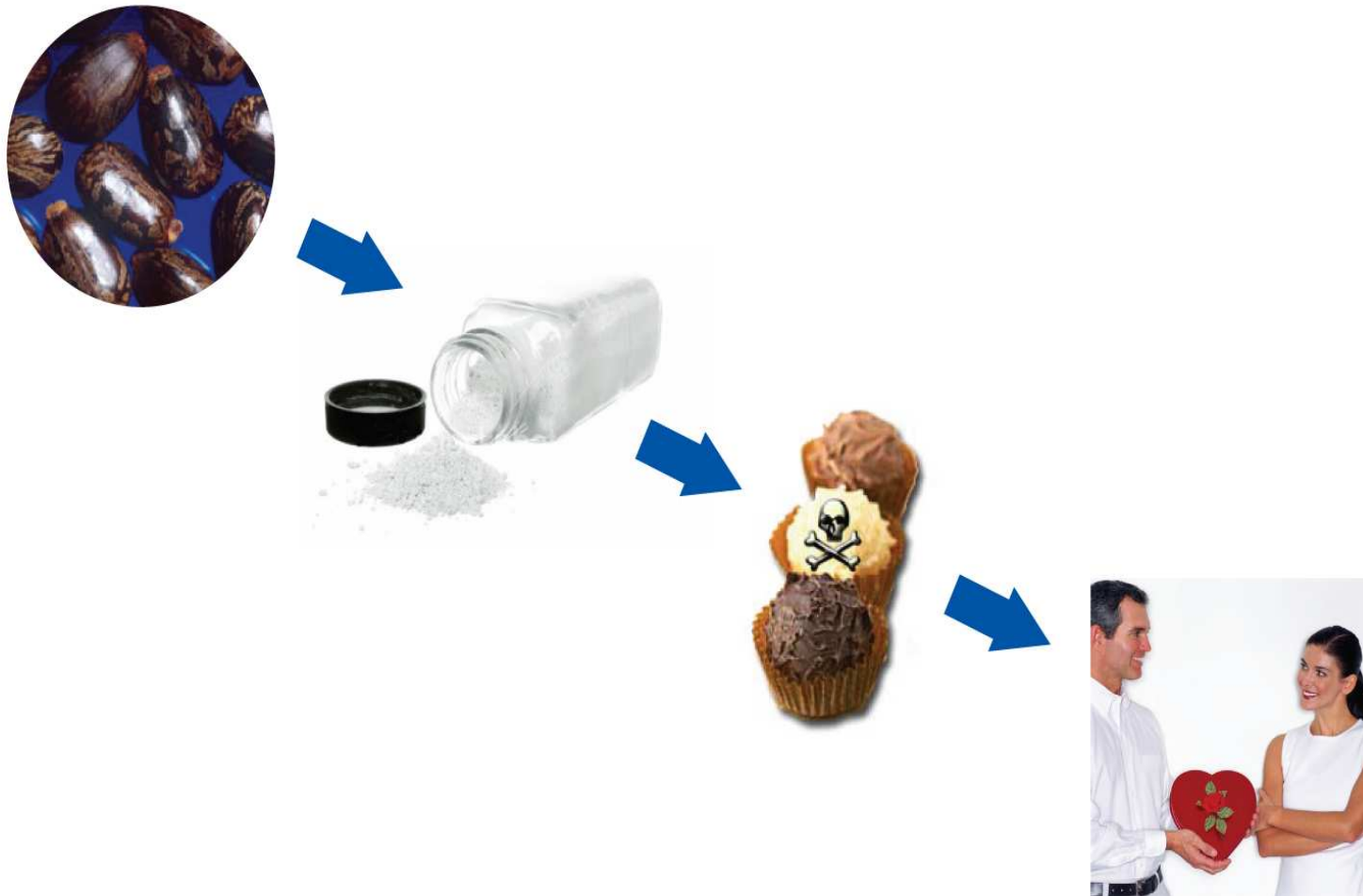


# Bioforensic Characterization of *B. anthracis*



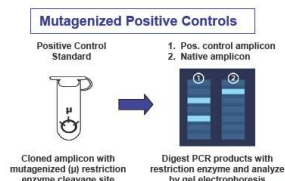
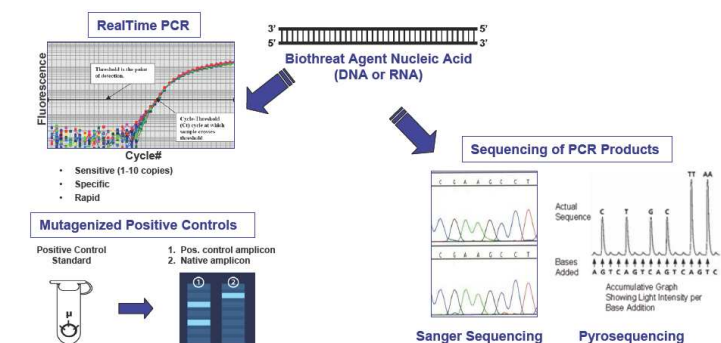
Anticipated Results	1 spore count (cfu/g)	5 antibiotic resistance x 20	9 spore shape, quality	13 genetic engineering
	2 viability/survivability	6 aerobic <i>in vitro</i> culture	10 particle size	14 gene identification
	3 plasmids pX01 pX02 +/-	7 protective antigen (PA)	11 strain identification	15 complete sequence
	4 specific to <i>B. anthracis</i>	8 polysaccharide & capsule	12 specific genes +/-	

# Ricin as a Biocrime Agent – An “Inappropriate Valentine’s Day Present”



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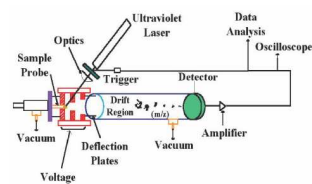
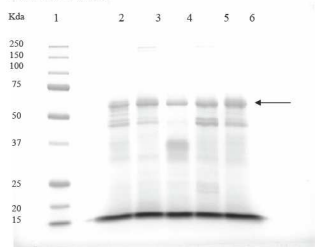
# Identification and Characterization of Toxins



Confirm Presence of Ricin Toxin Gene Sequence in Sample

**Molecular Identification**

Toxin protein identified by mass on SDS-PAGE



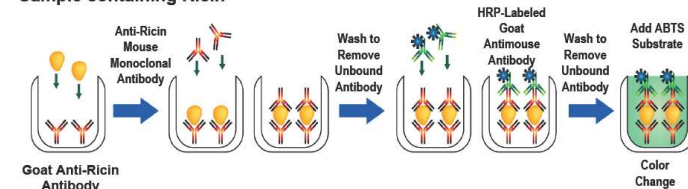
- Samples of appropriate MW excised from an SDS PAGE gel
- Excised sample digested with Trypsin
- Trypsinized sample analyzed by MALDI-TOF Mass Spectroscopy and matched to toxin peptide library

**Mass Spectroscopic ID**

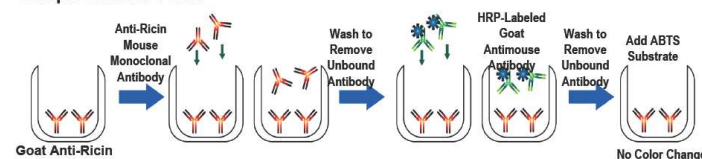


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Sample containing Ricin

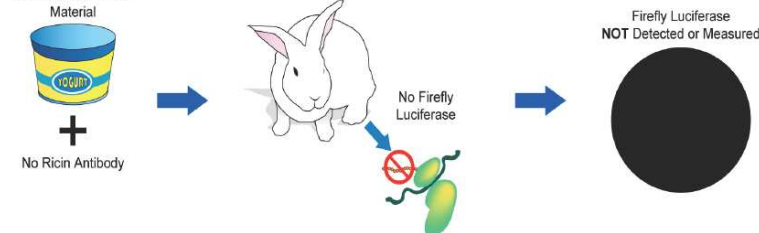


Sample without Toxin

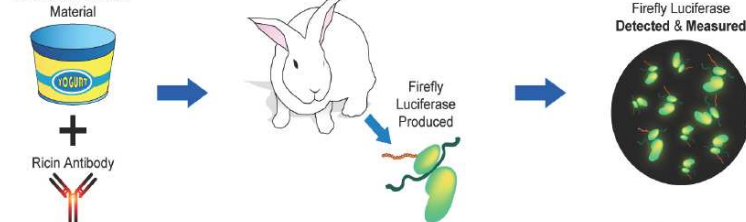


**Antigen Capture ELISA**

Ricin Contaminated Material



Ricin Contaminated Material

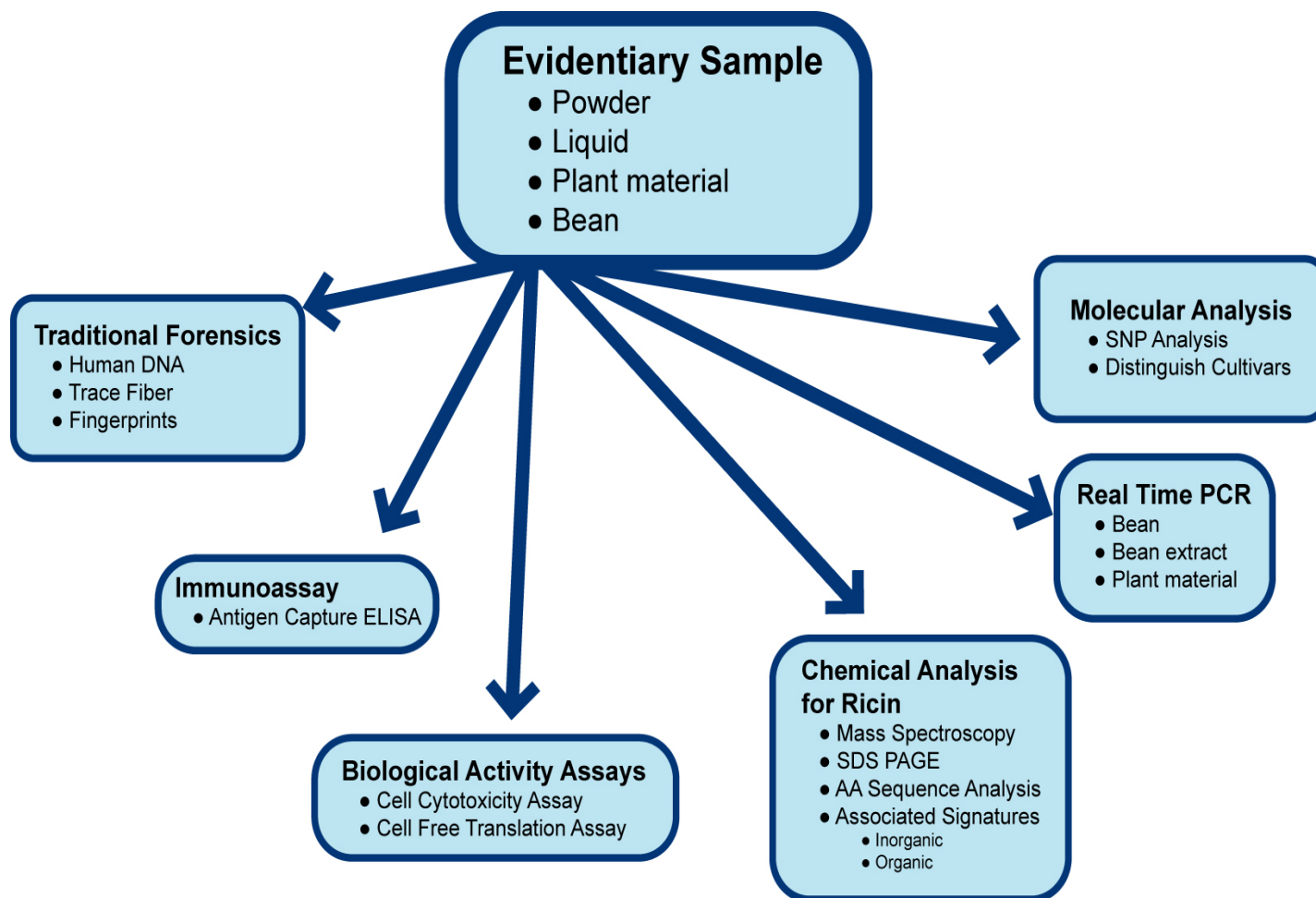


**Cell Free Translation Assay**



**BNBI**  
Battelle National  
Biodefense  
Institute

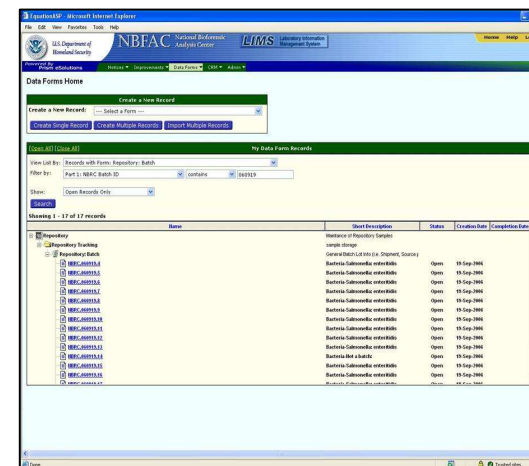
# Analytical Scheme for Evidence Suspected to Contain Ricin Toxin



# The National Bioforensic Repository Collection (NBRC)



- The NBRC is a Columbus, OH-based collection of bioanalytical standards
- Initiated in October of 2005, the project entails the receipt, storage and distribution of mission-critical, and often rare, high-value biological materials



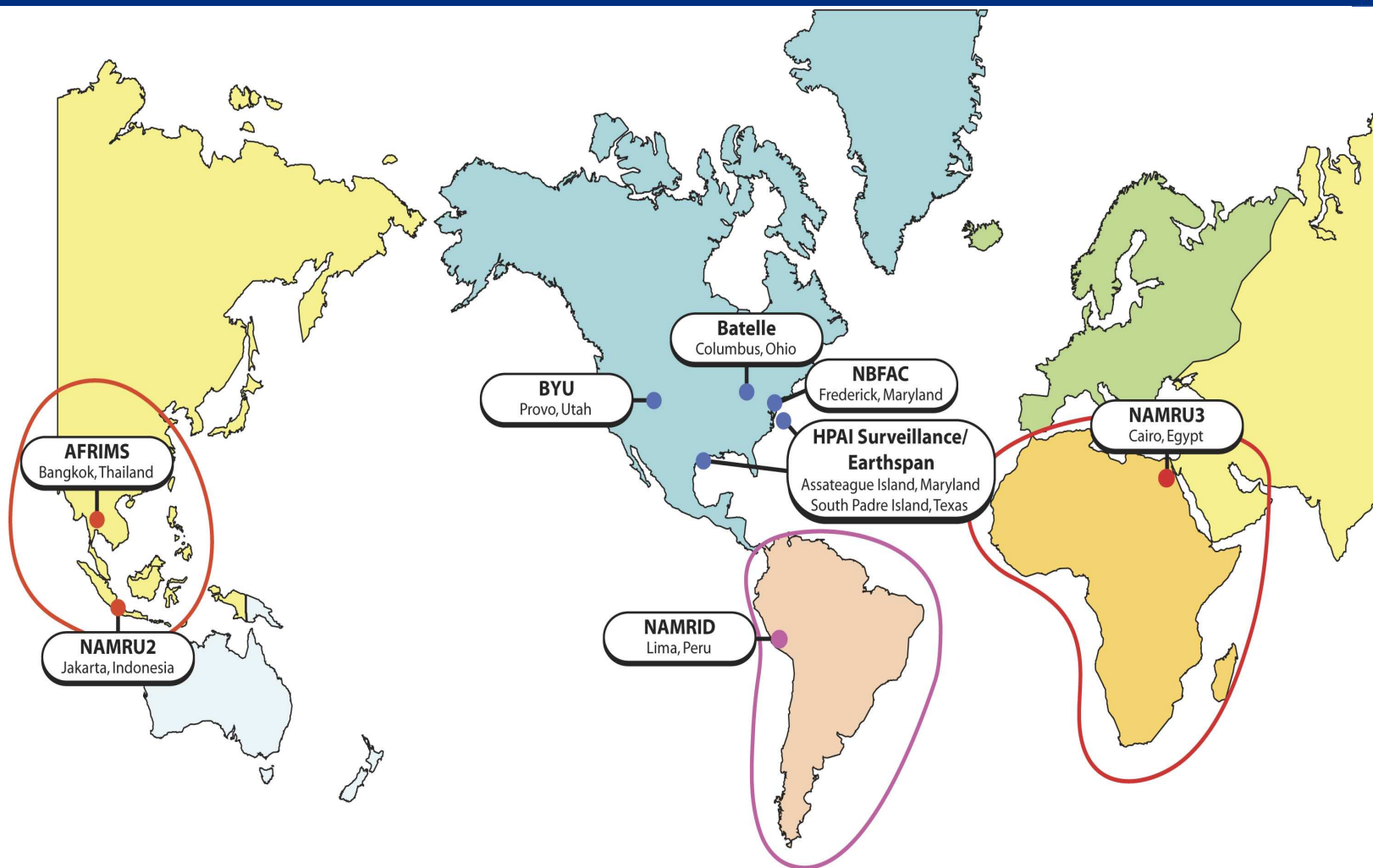
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**NBRC Operations**





# NBFAC Repository Collection Activities



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# Benefits of NBFAC

- Provides a national, dedicated bioforensic capability that establishes national standards and provides diverse forensic analytical capabilities through a centralized “Hub” and “Spoke” partner laboratory network
- Develops, evaluates, and validates bioforensic analyses for human, animal and plant (planned for the future) biological threat agents
- Provides validated data from the analysis of biological agents and related evidence that generates investigative leads for attribution for law enforcement and Homeland Security



# Homeland Security



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# Backup Slides

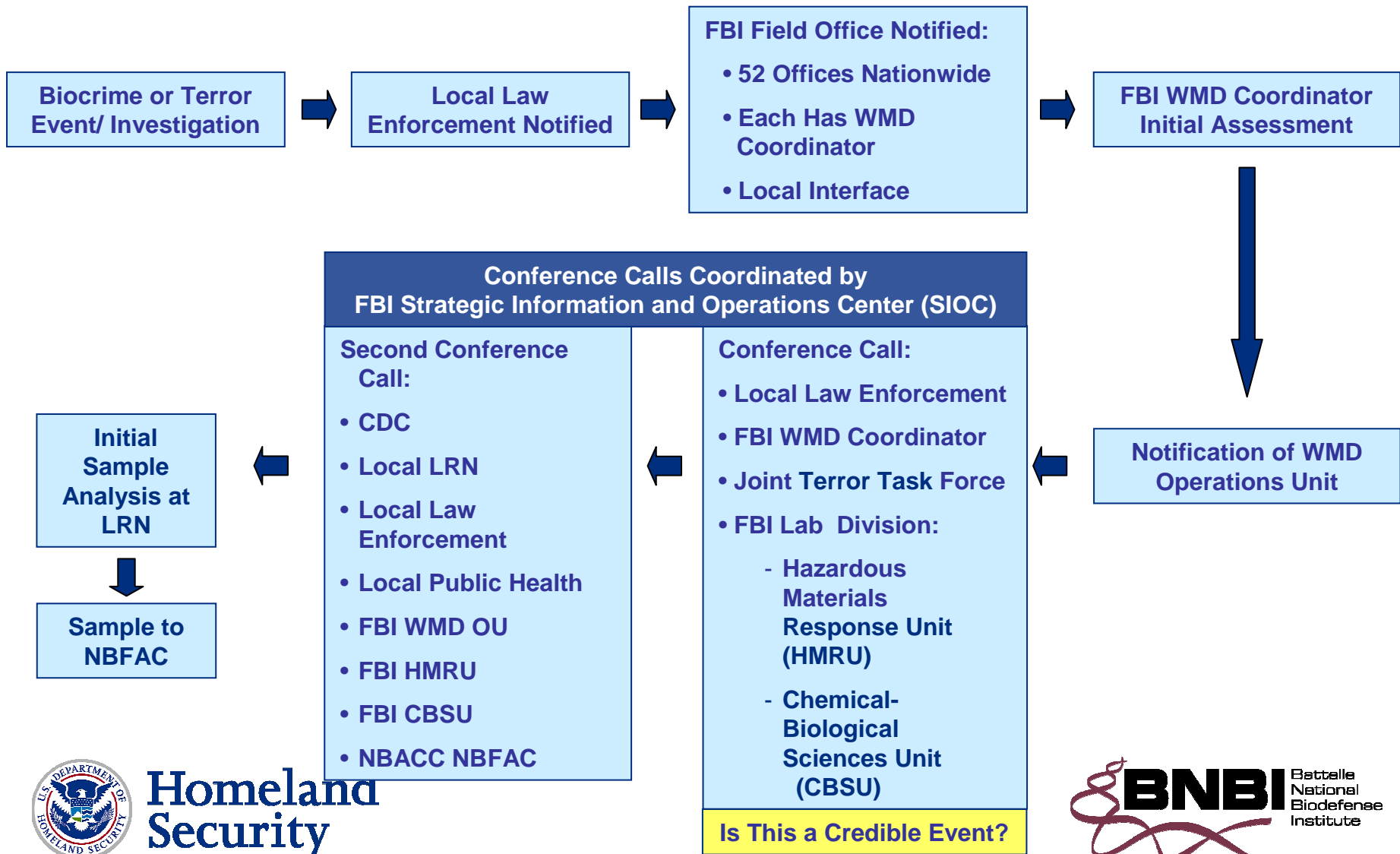
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# Biocrime/Terror Investigation or Event Scenario



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